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## Supplementary L-hydroxyproline attenuates disuse muscle atrophy-induced brain dysfunctionsbrains (#357)

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### Introduction

People desire to extend the 'healthy life expectancy', since life-span has been dramatically extended by medical developments in a few decades. Disuse muscle atrophy (DMA) is induced by muscle immobility. Elderly people have a higher risk of DMA owing to decline of their motor activity. Additionally, DMA leads brain dysfunctions, which impairs the 'Quality of Life.' Thus, it is required the prophylaxis which is easy to introduce their daily habits for elderly people, such as 'nutritional therapy.' Collagen peptides containing L-hydroxyproline (Hyp) have various functions. Orally ingested collagen peptides are well absorptive to tissues, and free L-Hyp is retained in the blood for several hours. Accordingly, it is presumed that L-Hyp is delivered more effectively to the brain than collagen peptides. In the present study, the effect of L-Hyp on brain dysfunction induced by DMA was evaluated from the perspectives of behavioral phenotypes and brain metabolism.

### Methods

The experimental procedure is shown in Fig. 1. Male 7 week-old ICR mice were divided into 3 groups: skin incised mice supplemented with distilled water (CON), muscle-atrophied mice supplemented with distilled water (ATR) and muscle atrophied mice supplemented with L-Hyp solution (Hyp). Muscle atrophied mice was cut their right ischiadic nerves. After the surgery, mice were orally administered either distilled water (10 ml/kg) or L-Hyp solution (5 mmol/10 ml/kg) once a day for 20 days. On Day 21, the open field test (OFT) was performed. Briefly, mice were allowed to explore in a black square arena for 5 minutes. The escape latency from the center area was observed as an index of the anxiety-like behavior. After 24 hours, the object exploration test (OET) was also performed. Briefly, mice were allowed to re-explore the same apparatus where two identical objects were placed. The total number of exploration to the objects was observed as an index of the motivated-like behavior. The exploration behavior was defined as sniffing and touching with their hands. On Day 23, mice were sacrificed under the anesthesia and their hippocampi were rapidly dissected. On Day 21-23, the distilled water or L-Hyp solution was given 1 hour prior to each behavioral test and sampling. From the collected hippocampi, water-soluble metabolites were extracted and derivatized samples were applied to GC-MS. Obtained data were analyzed by MS-DIAL version 3.30 (*Tsugawa H, Nat. Methods, 2015*) and MetaboAnalyst 4.0 (*Chong J, Nucleic Acids Res., 2018*). The statistical analys was performed using ANOVA accompanied by Tukey-Kramer test or t test.

### Results

In the OFT, anxiety-like behavior was induced by DMA, however, L-Hyp supplementation attenuated its aggravation. In the OET, motivated-like behavior was significantly decreased by DMA. L-Hyp ingestion slightly recovered the motivational deficit attributed to DMA. Consequently, the behavioral dysfunctions induced by DMA were mildly prevented by the dietary L-Hyp. In metabolome analysis of hippocampus, many metabolites were altered between CON and ATR mice. The enrichment analysis revealed that various metabolic pathways were affected by DMA. Especially, the metabolic pathways associated with energy production were altered. Meanwhile, there were specifically alterations in three metabolites between ATR and Hyp mice. L-Hyp and glycolate in Hyp mice robustly higher than those in CON and ATR mice. Glycolate is metabolized from L-Hyp via glyoxylate and is the resultant final product of that pathway. It is speculated that L-Hyp was transferred into the brain in surplus and metabolized to glycolate. In addition, urate level in Hyp mice was significantly higher than those in CON and ATR mice. It is suggested that L-Hyp ingestion may attenuate the abnormal behaviors mediated by urate increase because urate has a neuroprotective effect.

### Conclusion

In the present study, it is investigated the prevention effect of L-Hyp for brain dysfunctions associated with DMA. L-Hyp supplementation attenuated anxiety-like behavior and motivational deficit induced by DMA via the increase of urate which has a neuroprotective effects in the hippocampus. Consequently, it was suggested that supplemental L-Hyp ingestion is effective to prevent the brain dysfunctions in elderly people with DMA.

Notes

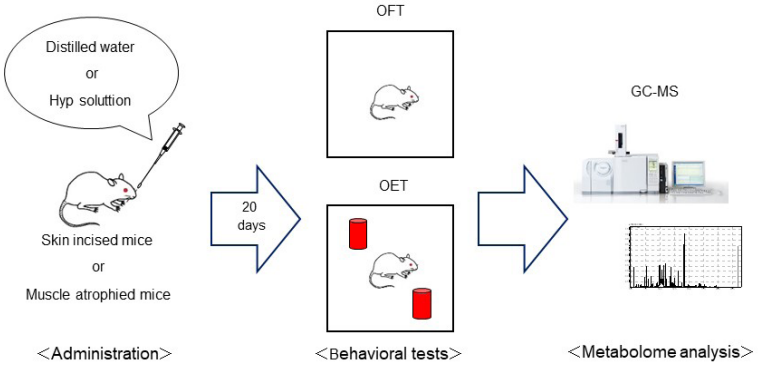


**Fig. 1 Experimental procedure**
Operated mice were administered distilled water or Hyp solution for 20 days. On Day 21 and 22, OFT and OET were performed to evaluate anxiety-like behavior and motivated-like behavior. On Day 23, their hippocampi were collected and subjected to metabolome analysis.

Notes